regulation of the basal L-type Ca^{2+} current by NO-cGMP cascade using the patch clamp method.
In the presence of 1 mM L-arginine in all experimental media the activation of NO-cGMP cascade by 5 mM L-arginine induced a steady suppression of L-type Ca^{2+} current amplitude on average by 30% in all experiments, which has not been observed previously. In the presence of 1 μM 7NI (NOS blocker) or KT5823 (PKG blocker), addition of 5 mM arginine had no marked effect on the amplitude of L-type Ca^{2+} current.
Next we investigated whether addition of 5 mM L-arginine affects the activation of α_2-adrenoceptors. It was shown that in the presence of α_2-adrenoceptor antagonists - yohimbine (10 μM) and rauwolscine (10 μM), 5 mM of arginine had no effect on the amplitude of L-type Ca^{2+} current. Then activity of some key components of the cascade activating NO synthase through α_2-adrenoceptors was checked. We showed that in the presence of 100 nM wortmannin (PIP_3 kinase blocker) or 0,5 μM Akt1/2 inhibitor (PKB bloker) 5 mM of L-arginine had no marked effect on L-type Ca^{2+} current.
Thus we have shown that extra addition of L-arginine affects the amplitude of L-type Ca^{2+} current and related with the activation of α_2-adrenoceptors followed by an increase in NOS activity.

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Activation of Na^+-Dependent Potassium Currents by Persistent Sodium Currents
Gonzalo Budelli, Travis Hage, Aguan Wei, Patricio Rojas,
Yuh-Jiin Ivy Jong, Karen O'Malley, Lawrence Salkoff.
Department of Anatomy and Neurobiology, Washington University School of Medicine, St Louis, MO, USA.
The Hodgkin-Huxley model of delayed repolarization of the sodium-dependent action potential assigns a voltage-dependent delayed rectifier K^+ current for repolarization. However, here we show that one of the largest components of the delayed rectifier current in many mammalian neurons has gone unnoticed and is due to a Na^+-activated-K^+-current/Persistent-Na^+-current coupled system. Previous studies of potassium conductances in mammalian neurons may have overlooked this large outward component because the sodium channel blocker TTX is typically used in such studies; we find that, in addition to blocking sodium currents, TTX also eliminates this delayed rectifier component as a secondary consequence. We unexpectedly found that the activity of the persistent inward sodium current (persistent I_{Na}) at cell resting potentials is the essential factor in activating the Na^+-dependent (TTX-sensitive) delayed rectifier current. The persistent I_{Na} apparently maintains Na^+-activated K^+ channels in a ''primed'' state so that, upon depolarization, they carry a delayed outward conductance. Persistent I_{Na} appears to raise the local concentration of Na^+ in the vicinity of Na^+-activated K^+ channels to higher levels than that of the bulk cytosol, possibly because of a submembrane diffusion-restricted space variously referred to in the literature as an ''unstirred'' layer" or ''fuzzy'' space". We showed that ''depleting'' or ''filling'' this diffusion-restricted space by the action of persistent I_{Na} requires several seconds at cell resting potentials. Using siRNA techniques we identified SLO2.2 (Slack) channels as carriers of the Na^+-dependent delayed rectifier current. These findings of a previously unseen K^+ conductance involving a finely tuned partnership linking persistent I_{Na} and Na^+-activated K^+ channels, have far reaching implications for many neurons of the mammalian brain. Studies of ''up-down'' states of neuronal excitability, spike adaptation, synaptic integration, and other aspects of neuronal physiology may have to be reexamined taking this system into account.

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Uptake Of S100A1 And Augmentation Of Cav1 Channel Current And Action Potential Duration In Sympathetic Ganglion Neurons
Erick O. Hernández-Ochoa, Benjamin L. Prosser, Nathan T. Wright, Minerva Contreras, David J. Weber, Martin F. Schneider.
University of Maryland, Baltimore, MD, USA.
S100A1, a ~20 kD dimeric Ca^{2+}-binding protein of the EF-hand type, is highly expressed in cardiomyocytes and is considered an important regulator of heart function. During ischemia, cardiomyocytes release S100A1 to the extracellular space. Although the effects of extracellular S100A1 have been documented in cardiomyocytes, it is unclear whether S100A1 exerts modulatory effects on other tissues in close proximity with cardiac cells. Neurons from the cervical ganglion chain extend postganglionic axons that innervate the heart. Therefore, we sought to investigate the effects of exogenous S100A1 on Ca^{2+} signals and electrical properties of principal sympathetic ganglion neurons (SGNs) from the superior cervical ganglion. Immunostaining and western blot analysis did not detected any endogenous S100A1 in SGNs from normal mice. Cultured adult SGNs took up fluorescent S100A1 when it was present in the extracellular media. Inside the cell exogenous fluorescent S100A1 localized primarily in a punctuate pattern throughout the cytoplasm and axoplasm, but was excluded from the nuclei. Time lapse imaging and FRAP experiments reveal axonal transport of S100A1 puncta, presumably endosomes. In compartmentalized (Campenot) SGN cultures, axonal projections were capable of uptake and transport of S100A1 towards the neuronal somas. Exogenous S100A1 enhanced Cav1 channel currents, increased the amplitude of action potential-evoked Ca^{2+} transients and prolonged the action potentials. Our results, showing enhanced somatic Ca^{2+} entry, larger cytosolic Ca^{2+} transients, and prolongation of action potential duration, suggest the hypothesis that S100A1 released from heart muscle cells may be taken up by sympathetic neurons, leading to an increase of sympathetic output to the heart. Supported by NIH, Grants R01-N5042839, R01-AR055099.

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Direct Interaction Of A Small Molecule Modulator With G551D-CFTR, A Cystic Fibrosis Causing Mutation Associated With Severe Disease
Stan Pasyk[1,2], Canhui Li[2], Mohabir Ramjeesingh[2], Christine E. Bear[2,1].
[1]University of Toronto, Toronto, ON, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada.
CFTR is a member of the ATP Binding Cassette (ABC) superfamily of membrane proteins, and a disease-causing missense mutation within the ABC signature sequence; G551D-CFTR, exhibits defective phosphorylation and ATP dependent channel gating. Studies of the purified and reconstituted G551D-CFTR protein revealed that faulty gating is associated with defective ATP binding and ATPase activity, reflecting the key role for G551 in these functions. Recently, high-throughput screens of chemical libraries led to identification of modulators which enhance channel activity of G551D-CFTR. However, the molecular target(s) for these modulators and their mechanism of action remains unclear. In the present study, we evaluated the mechanism of action of one small molecule modulator: VRT-532, identified as a specific modulator of CF causing mutants. First, we confirmed that VRT-532 caused a significant increase in channel activity by G551D-CFTR using a novel assay of CFTR function in inside-out membrane vesicles. This versatile assay of iodide conductance enables the study of large populations of mutant proteins in a cell-free system, removed from other confounding cellular proteins. Biochemical studies of purified and reconstituted G551D-CFTR revealed that potentiation of the ATPase activity by VRT-532 is mediated by enhancing the affinity of the mutant for ATP. Interestingly, VRT-532 did not affect the ATPase activity of the wild type CFTR, supporting the idea that this compound corrects the specific molecular defect in this mutant. To summarize, these studies provide direct evidence that this compound binds to G551D-CFTR to rescue its specific defect in ATP binding and hydrolysis. These studies provide rationale for using G551D as a tool for identifying the binding site of VRT-532. Studies supported by the Canadian Cystic Fibrosis Foundation and by Cystic Fibrosis Foundation Therapeutics, Inc.

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Oscillation of the Membrane Potential of T-cells Forming Immunological Synapse
Ferenc Papp[1], Ágnes Tóth[2], Orsolya Szilágyi[2], Sándor Damjanovich[2], **Gyorgy Panyi**[2].
[1]University of Debrecen, Cell Biology and Signaling Research group of the HAS, Debrecen, Hungary, [2]University of Debrecen, Debrecen, Hungary.
Ion channels important in the activation of T-cells, the voltage-gated K^+ channel Kv1.3, the Ca^{2+}-activated K^+ channel (IKCa1) and the Calcium release-activated Ca^{2+} channel (CRAC) are all recruited in the signaling platform of T-cells called Immunological Synapse (IS). Kv1.3 and IKCa1 channels set the resting membrane potential of T cells between -50 and -60mV. This membrane potential is maintained in spite of the depolarizing Ca^{2+} influx through CRAC channels during T-cell activation. We hypothesized that the strategic recruitment of the channels into the IS gives rise to a unique membrane potential response of T-cells conjugated in an IS.
Membrane potential was measured using patch-clamp technique in current-clamp mode. The pipette filling solution was based on KCl supplemented with 0.3g/l Nystatin to perform perforated patch-clamp and $5*10^{-4}$ % Fluorescein to validate the perforated-patch recording mode. IS was formed between the antigen presenting murine B-cell line CH-12 and D10 murine T-cells specific for the antigen conalbumin. D10 cells express PKC-theta-GFP to indicate the formation of an IS.
The membrane potential of D10 cells conjugated in IS (''conjugated'') was the same as ''lonely'' D10 cells not forming an IS, being approximately -50 mV. Applying 150 mM K^+ external solution depolarized the membrane potential to 0 mV indicating the dependence of the membrane potential on K^+ channels. Injecting +10 - +20 pA current caused a depolarization and then produced oscillation in membrane potential of ''conjugated'' D10 cells whereas current injection caused simply depolarization in ''lonely'' D10 cells. The magnitude and frequency of the oscillations were 18.3 ± 2.1mV and 0.08 ± 0.01 s^{-1}, respectively. 50nM Charybdotoxin (blocker of Kv1.3 and IKCa1 channels) depolarized the membrane potential and cancelled the oscillatory membrane potential response. Supp: OTKA K 60740 and NK 61412.